



## ORIGINAL ARTICLE

# Distribution of Virulence Genes and Their Association of Serotypes in Pathogenic *Escherichia coli* Isolates From Diarrheal Patients in Korea

Seung-Hak Cho, PhD, Kyung-Hwan Oh, PhD, Seong-Han Kim, PhD, Hee-Bok Oh, PhD, Mi-Sun Park, PhD\*

Division of Enteric Bacterial Infections, Center for Infectious Diseases, Korea National Institute of Health, Seoul, Korea

Received: Sep 7, 2010  
Revised: Oct 8, 2010  
Accepted: Oct 21, 2010

**KEY WORDS:**

diarrhoeal patients;  
pathogenic *Escherichia coli*;  
serotypes;  
virulence factors

**Abstract  
Objectives**

To characterise the genetic and serological diversity of pathogenic *Escherichia coli*, we tested 111 *E coli* strains isolated from diarrhoeal patients in Korea between 2003 and 2006.

**Methods**

The isolates were tested through polymerase chain reaction (PCR) and slide agglutination method for the detection of virulence genes and serotypes, respectively. To compare the expression of Shiga toxin (*stx*)-1 and *stx2* genes, real-time quantitative reverse-transcriptase PCR and rapid expression assay, reversed-passive latex agglutination, were performed.

**Results**

Forty-nine Shiga toxin-producing *E coli* (STEC) strains and 62 non-STEC strains, including 20 enteropathogenic *E coli*, 20 enterotoxigenic *E coli*, 20 enter-oaggregative *E coli*, and 2 enteroinvasive *E coli* were randomly chosen from the strains isolated from diarrhoeal patients in Korea between 2003 and 2006. PCR analysis indicated that locus of enterocyte effacement pathogenicity island, that is, *eaeA*, *espADB*, and *tir* genes were present in STEC, enteropathogenic *E coli*, and enteroinvasive *E coli*. Quorum sensing-related gene *luxS* was detected in most of pathogenic *E coli* strains. Major serotypes of the STEC strains were O157 (26%) and O26 (20%), whereas the non-STEC strains possessed various serotypes. Especially, all the strains with serotype O157 carried *stx2* and the tested virulence factors. Of the STEC strains, the data of real-time quantitative reverse-transcriptase PCR and reversed-passive latex agglutination tests showed that messenger RNA- and protein expression of *stx2* gene were higher than those of *stx1* gene.

\*Corresponding author. Division of Enteric Bacterial Infections, Center for Infectious Diseases, Korea National Institute of Health, 194, Tongil-ro, Eunpyeong-gu, Seoul 122-701, Korea.  
E-mail: pmsun63@korea.kr

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Conclusion

Our results provide the epidemiological information regarding the trend of STEC and non-STEC infections in the general population and show the fundamental data in association of serotypes with virulence genes in diarrhoeagenic *E coli* strains from Korea.

## 1. Introduction

Diarrhoea is an extraordinarily common disease with worldwide distribution, and diarrhoeagenic *Escherichia coli* is an important bacterium to cause diarrhoeal disease.<sup>1,2</sup> In a surveillance of bacterial pathogens associated with acute diarrhoeal disease in the Republic of Korea, it has been found that pathogenic *E coli* are frequently isolated from diarrhoeal patients (around 20%), and enterohaemorrhagic *E coli* (EHEC) accounts for ca. 2% among the isolated pathogenic *E coli*.<sup>3</sup> The pathogenic strategies of the diarrhoeagenic *E coli* strains exhibit remarkable variety. Three general paradigms have been described by which *E coli* may cause diarrhoea;<sup>2</sup> each is described in detail in the appropriate section below: enterotoxin production [enterotoxigenic *E coli* (ETEC) and enteroaggregative *E coli*, (EAEC)], invasion [enteroinvasive *E coli* (EIEC)], and intimate adherence with membrane signalling [enteropathogenic *E coli* (EPEC) and EHEC].<sup>4–7</sup>

The major virulence factor, which is a defining characteristic of EHEC, is Shiga toxin (Stx). Shiga toxin-producing *E coli* (STEC) strains produce one or both of two major types of shiga toxin, designated Stx1 and Stx2, and the production of the latter is associated with an increased risk of developing haemolytic-uremic syndrome.<sup>1,8</sup> The prototypical Stx1 and Stx2 toxins have 55% and 57% sequence homology in the A and B subunits, respectively.<sup>2,6</sup> ETEC strains are identified by the ability to produce enterotoxins, heat-labile toxin (LT), heat-stable toxin (ST), and surface adhesions known as colonization factors.<sup>7</sup> Atypical EPEC strains would possess the attaching and effacing (*eae*) gene that correlates with possession of 35-kb locus of enterocyte effacement (LEE) pathogenicity island encoding *eae*.<sup>9</sup> LEE encodes a Type III secretion system and *E coli* secreted proteins through Type III secretion system, which deliver effector molecules to the host cell and disrupt the host cytoskeleton.<sup>4,5,10,11</sup> LEE also carries *eae*, which encodes an outer membrane protein (intimin) required for intimate attachment to epithelial cells; therefore, *eae* has been used as a convenient diagnostic marker for LEE-positive EPEC strains.<sup>9</sup> EAEC strains produce enteroaggregative heat-stable enterotoxin (*east*)-1 encoded by *east1* gene.<sup>12,13</sup> Furthermore, other virulence factors of pathogenic *E coli* for the diarrhoeagenic infections, such as EHEC hemolysin (*E-hly*), *espADB* (Type III secretion proteins), and *tir*

(translocated intimin receptor), have been found in diarrhoeagenic *E coli* strains. Quorum sensing is a mechanism of cell-to-cell signalling involving the production of hormone-like compounds called auto-inducers.<sup>14,15</sup> Regulation of LEE genes by quorum sensing is reported.<sup>16</sup> When these molecules reach a certain concentration threshold, they interact with bacterial regulatory proteins, thereby controlling gene regulation mechanism in both gram-negative and gram-positive bacteria.<sup>14,15</sup> The gene encoding the auto-inducer-2 synthetase was cloned, sequenced, and named *luxS*. Recently, the global regulation of virulence factors was investigated in clinical EHEC isolate in Korea by creating a  $\Delta luxS$  mutant strain.<sup>17</sup>

To obtain the epidemiological information on various virulence factors in different pathogenic *E coli* groups, we characterized genetic diversity of *E coli* strains isolated from diarrhoeal patients in Korea from 2003 to 2006 through the surveillance system performed by Laboratory of Enteric Infections in Korea National Institute of Health.

## 2. Materials and Methods

### 2.1. *E coli* strains isolated from stool specimen

A total of 111 *E coli* strains isolated from the stool of diarrhoeal patients in Korea between 2003 and 2006 were investigated in this study. Bacteria were plated on MacConkey agar. All isolates were biochemically identified with the API20E system (bioMérieux, Marcy l'Etoile, France).

### 2.2. Detection of virulence genes by PCR

Bacteria was directly inoculated into 3 mL of Luria-Bertani broth for enrichment and incubated overnight at 37°C under shaking conditions. After incubation, enriched broth culture was centrifuged at 13,000 rpm (Sorvall® Biofuge Pico, Germany) for 1 minute, and the pellet was heated at 100°C for 10 minutes. After centrifugation of the lysate, 5 µL of the supernatant was used in the polymerase chain reactions (PCRs). To identify virulence genes, PCR assays were performed using the primers shown in Table 1. PCR assays were carried out in a 50 µL volume with 2U DNA *Taq* polymerase (Takara Ex *Taq*™, Japan) in a thermal cycler (PTC-100; MJ Research, Watertown, MA, USA) under

**Table 1** Primers used in this study

Target gene	Primer sequence (5' to 3')	Size of the PCR product (bp)
Shiga toxin 1 ( <i>stx1</i> )	CGTACGGGGATGCAGATAAATCGC CAGTCATTACATAAGAACGCCCCAC	210
Shiga toxin 2 ( <i>stx2</i> )	GTTCTGCGTTTTGTCACTGTACAC GTCGCCAGTTATCTGACATTCTGG	326
Heat-stable enterotoxin ( <i>east1</i> )	ATGCCATCAACACAGTATATCCG TCAGGTCGCGAGTGACGGCTTT	119
Attaching and effacing ( <i>eaeA</i> )	ATGCTGGCATTGTTGTCAGGTCGG TGACTCATGCCAGCCGCTCATGCG	233
Heat-labile toxin ( <i>lt</i> )	GATCACGCGAGAGGAACACAAACC ATCTGTAACCATCCTCTGCCGGAG	366
Heat-stable toxin ( <i>st</i> )	CTTTCCCCTCTTTAGTCAGTC CACAGGCAGGATTACAACAAAGT	302
Invasion-associated locus ( <i>ial</i> )	GTTGCGCTTGATGGGTGGGGTATC GAAATGTCCATCAAACCCCACTC	356
Hemolysin ( <i>hlyA</i> )	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	519
Type III secretion protein ( <i>espA</i> )	GTTTTTCAGGCTGCGATTCT AGTTTGGCTTTTCGCATTCTT	187
Type III secretion protein ( <i>espD</i> )	AAAAAGCAGCTCGAAGAACA CCAATGGCAACAACAGCCCA	145
Type III secretion protein ( <i>espB</i> )	GCCGTTTTTGAGAGCCAGAA AAAGAACCTAAGATCCCCA	106
Translocated intimin receptor ( <i>tir</i> )	GCTTGCAGTCCATTGATCCT GGGCTTCCGTGATATCTGA	107
Autoinducer-2 synthetase ( <i>luxS</i> )	GTCGACGCCGCTGATACCGAACCG GTCGACGCGGTGCGCACTAAGTACAA	792

PCR = polymerase chain reaction; bp = base pair.

the following conditions: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 1 minute, 72°C for 1 minute, and final cycle 72°C for 5 minutes. Amplified PCR products were analysed by gel electrophoresis in 2% agarose gels stained with ethidium bromide, visualized with ultraviolet illumination, and imaged with the Gel Doc 2000 documentation system (Bio-Rad, Hercules, CA, USA).

### 2.3. Total RNA isolation

RNA extracts of the strains were prepared using the Qiagen RNeasy midi-prep kit and RNA BacteriaProtect (QIAGEN Co. Ltd, Germany) according to the manufacturer's instructions for gram-negative bacteria.

#### 2.3.1. Amplification of target genes by real-time reverse-transcriptase-PCR and analysis

For quantitative real-time reverse-transcriptase-PCR (qPCR), the 20 µL reaction mixture was prepared by 2 µL of total RNA, 0.6 µM of each primer, and reference dye Synergy Brands Inc. Green. The number of copies was calculated, and dilutions ranging from 100 pg to 100 ng copies of this standard were prepared in a Tris-

ethylenediaminetetraacetic acid buffer. Aliquots of these dilutions were frozen at -20°C. Throughout this study, the Quantitect Synergy Brands Inc. Green master mix kit (Qiagen, Valencia, CA, USA) was used for all reactions with qPCR. The parameters for qPCR included 30 minutes incubation at 50°C for converting messenger RNA (mRNA) to complementary DNA. Subsequent amplification of complementary DNA was carried out by using an initial cycle of 95°C for 10 minutes followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds. The final extension was carried out at 72°C for 2 minutes. For the quantification, of *stx1*, *stx2*, and *gapA* (housekeeping gene) mRNA, a negative control, consisting of nuclease-free water in place of template DNA, was included in each PCR run. The primers used for real-time PCR were F 5'-GATGATCT-CAGTGGGCGTTC-3', R 5'- CAACTCGCGATGCAT-GATG-3' (*stx1*), F 5'-CGCACTGTCTGAACTGCTC-3', R 5'- TCGCCAGTTATCTGACATTC-3' (*stx2*), F 5'-ACTTCGACAAATATGCTGGC-3', R 5'- CGGGAT-GATGTTCTGGGAA-3' (*gapA*). Reaction conditions for amplification and parameters for fluorescence data collection were programmed into an Opticon Monitor Software package 1.4 (DNA engine Opticon 2; MJ Research, Watertown, MA, USA). All assays were

$$\text{Quantitative (expression) ratio} = \frac{2^{\Delta C_{t\text{target gene (control-test)}}}}{2^{\Delta C_{t\text{endogenous gene (control-test)}}}}$$

performed in triplicate. The threshold cycle ( $C_t$ ) values of the known standards were plotted versus the logarithm of the concentration of each standard creating a standard curve. Samples of unknown concentration were plotted onto the standard curve to calculate their concentration. Normalization of the quantification results from *stx1* and *stx2* was performed by the incorporation of the quantification results of *gapA* mRNA into the following equation:

Here,  $\Delta C_t$  endogenous gene (control – test) =  $C_t$  value of the endogenous gene (*gapA*) with the control RNA –  $C_t$  value of the endogenous gene (*gapA*) from the sample RNA. Also,  $\Delta C_t$  target gene (control – test) =  $C_t$  value of the target gene (*stx1* or *stx2*) with the control RNA –  $C_t$  value of the target gene (*stx1* or *stx2*) with the test RNA.

#### 2.4. Reversed-passive latex agglutination test for the detection of Shiga toxin

The production of Stx1 and Stx2 by the isolates was determined by using a reversed-passive latex agglutination (RPLA) kit (VTEC-RPLA; Denka Seiken Co., Ltd., Tokyo, Japan) after having been grown and shaken in 5 mL of Tryptone Soya Broth overnight at 37°C. Of this suspension, 1 mL was centrifuged for 20 minutes at 13,000 rpm. The titre of the supernatant was determined in the verotoxin-producing *E. coli*-RPLA test according to the manufacture's instructions up to 1:256. All STEC strains were tested for the production of Stx1 and Stx2. Titres lower than 1:2 were interpreted as a negative control.

#### 2.5. Serotyping of O antigen

The presence of O antigens was determined by slide agglutination with the method of Guinée et al.<sup>18</sup> using all available O antisera (O1–O181). All antisera were absorbed with the corresponding cross-reacting antigens to remove the non-specific agglutinins.

### 3. Results

#### 3.1. Diversity of virulence genes in pathogenic *E. coli* isolates from diarrhoeal patients

To characterize genetic diversity of virulence factors, 111 pathogenic *E. coli* strains, 49 STEC strains, and 62 non-STEC strains, were chosen from the strains isolated from diarrhoeal patients in Korea between 2003 and 2006. All the strains were tested through PCR method with the primers as shown in Table 1. As shown in Table 2, in the genomic DNA of the 49 STEC strains, 22 strains were *stx1* positive and 17 were *stx2* positive. Ten possessed both *stx1* and *stx2* genes.

To detect major virulence genes for pathogenic *E. coli*, *eaeA* gene, major virulence factor of EPEC, *lt* and *st* genes for ETEC, *east1* gene for EAEC, and invasion-associated locus gene for EIEC were analysed in the STEC and non-STEC strains by PCR with the specific primers for these genes. Among the non-STEC strains, 20 EPEC, 20 ETEC, 20 EAEC, and 2 EIEC strains were randomly chosen among the pathogenic *E. coli* strains isolated from diarrhoeal patients in Korea. The *eaeA* gene was present in EPEC, STEC, and EIEC strains.

**Table 2** Distribution of virulence genes in pathogenic *Escherichia coli* from diarrhoeal patients

Pathogen (n) Major genes (n)		Virulence genes (%)										
		<i>eaeA</i>	<i>lt</i>	<i>st</i>	<i>east1</i>	<i>ial</i>	<i>hlyA</i>	<i>espA</i>	<i>espD</i>	<i>espB</i>	<i>tir</i>	<i>luxS</i>
STEC (49)	<i>stx1</i> only (22)	12 (55)	0 (0)	0 (0)	3 (14)	0 (0)	14 (64)	14 (64)	13 (59)	14 (64)	13 (59)	22 (100)
	<i>stx2</i> only (17)	13 (77)	1 (6)	0 (0)	2 (12)	0 (0)	13 (77)	11 (65)	13 (77)	11 (65)	12 (71)	16 (94)
	<i>stx1</i> and 2 (10)	7 (70)	1 (10)	0 (0)	3 (30)	0 (0)	8 (80)	6 (60)	7 (70)	5 (50)	5 (50)	10 (100)
EPEC (20)	<i>eaeA</i> (20)	20 (100)	2 (10)	0 (0)	5 (25)	0 (0)	2 (10)	11 (55)	20 (100)	20 (100)	11 (55)	11 (55)
ETEC (20)	<i>lt</i> (6)	0 (0)	6 (100)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (100)
	<i>st</i> (1)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
	<i>lt</i> and <i>st</i> (13)	0 (0)	13 (100)	13 (100)	13 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (100)
EAEC (20)	<i>east1</i> (20)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (70)
EIEC (2)	<i>ial</i> (2)	1 (50)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)

STEC = Shiga toxin-producing *E. coli*; EPEC = enteropathogenic *E. coli*; EAEC = enteroaggregative *E. coli*; ETEC = enterotoxigenic *E. coli*; EIEC = enteroinvasive *E. coli*.

**Table 3** Prevalence of serotypes in pathogenic *Escherichia coli* from diarrhoeal patients

Pathogen (n)	Serogroups (n)
STEC (49)	O157 (13), O26 (10), O103 (6), O111 (4), O121 (4), O55 (3), O91(3), O117 (2), O2 (1), O21 (1), O104 (1), O119 (1)
EPEC (20)	OUT* (8), O26 (2), O1 (1), O11 (1), O110 (1), O119 (1), O139 (1), O142 (1), O152 (1), O159 (1), O35 (1), O63 (1)
EAEC (20)	OUT (3), O169 (2), O20 (2), O153 (2), O158 (1), O18 (1), O8 (1), O124 (1), O114 (1), O25 (1), O166 (1), O15 (1), O146 (1), O5 (1), O51 (1)
ETEC (20)	O6 (8), O20 (3), O8 (2), O25 (2), OUT (2) O9 (1), O15 (1), O102 (1)
EIEC (2)	OUT (2)

STEC = Shiga toxin-producing *E. coli*; EPEC = enteropathogenic *E. coli*; EAEC = enteroaggregative *E. coli*; ETEC = enterotoxigenic *E. coli*; EIEC = enteroinvasive *E. coli*.

\*OUT, non-typeable O serotype.

The *lt* and *st* genes showed different prevalence in the pathogenic *E. coli* strains. The *lt* gene was found in various pathogenic *E. coli* groups: ETEC, STEC, EPEC, and EIEC; whereas the *st* gene was detected only in ETEC strains. Interestingly, the *east1* gene was also distributed in various pathogenic *E. coli* groups except EIEC strains. Eighty-five percent (17 of 20 strains) of ETEC strains harboured *east1* gene. This gene was detected in 16% of STEC and 25% of EPEC strains. However, invasion-associated locus gene was found only in EIEC strains (Table 2).

The prevalence of *hlyA*, *espADB*, *tir*, and *luxS* genes in STEC and non-STEC strains was determined in pathogenic *E. coli* isolates. Seventy-one percent (35 of 49 strains) of the STEC strains harboured the *hlyA* gene, whereas all the ETEC, EAEC, and EIEC strains were negative for this gene. Among the EPEC strains, only 10% were positive for this gene. LEE-associated genes, *espADB*, and *tir* genes were present in 61%–67% of STEC strains: *espA* (63%), *espD* (67%), *espB* (61%), and *tir* (60%). Interestingly, *espA* and *tir* genes in the EPEC strains were less prevalent than *espD* and *espB* genes. All EPEC strains harboured *espD* and *espB* genes, whereas *espA* and *tir* genes were present only in 55% of the strains. Among the ETEC and EAEC strains, LEE-

associated genes were not detected. These genes were found in the one of the two EIEC strains. Most of the pathogenic *E. coli* strains carried *luxS* gene. This gene was present in all ETEC strains and 97% of the STEC strains. The presence of this gene was similar in EPEC (55%), EAEC (70%), and EIEC (50%) strains (Table 2).

### 3.2. Serotyping of pathogenic *E. coli* isolates from diarrhoeal patients

As shown in Table 3, the O antigen was typeable for 95 strains, which could be classified into 38 different serotypes, and 15 strains were non-typeable. Among the STEC strains, 12 serotypes were identified. Major serotypes of the STEC strains were O157 (26%) and O26 (20%). Under the non-STEC strains, the EPEC, ETEC, and EAEC strains showed diversity of serotypes. However, the serotype of two EIEC strains was non-typeable.

### 3.3. Association of serotypes of pathogenic *E. coli* isolates with virulence genes

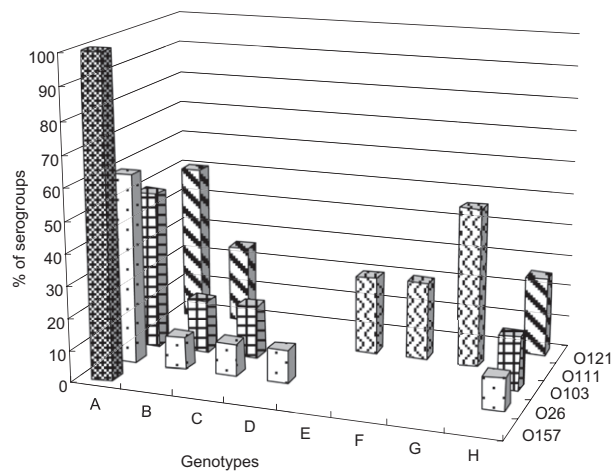
The serotypes of STEC strains were examined in association with virulence genes. The data of serotypes in association with *stx* genes were described in Table 4. The *stx1* gene was mostly distributed between the O26 (80%) and O103 (100%) serotypes. The *stx2* gene was present in the great majority of the O157 (100%) and O121 (100%) serotypes. As shown in Figure 1, all O157 strains harboured all the tested genes, that is, *eaeA*, *hlyA*, *espADB*, and *tir* genes. The serotypes that possessed all the genes described above were O26 (6 of 10 strains), O103 (three of six strains), and O121 (two of four strains), except for serotype O157 (Group A). Among the four O26 strains, *eaeA*-negative strain (Group B), *hlyA*-negative strain (Group B), *espD*-negative strain (Group C), and *eaeA*, *hlyA*, *espADB*, *tir*-negative strain (Group H) were found.

**Table 4** Serogroups in association with *stx* genes

Presence of <i>stx</i> genes (n)	Serogroups (n)
<i>stx1</i> only	O26 (8), O103 (6), O55 (3), O91 (2), O21 (1), O111 (1)
<i>stx2</i> only	O157 (7), O26 (2), O121 (4), O111 (1), O2 (1), O104 (1), O119 (1)
<i>stx1</i> and <i>stx2</i>	O157 (6), O111 (2), O117 (2) O91 (1)

*stx* = Shiga toxin gene.





**Figure 1** Distribution of virulence genes relative to serotypes of Shiga toxin-producing *Escherichia coli* (STEC) strains. A: *eaeA*, *hlyA*, *espADB*, *tir*; B: *eaeA*<sup>+</sup>, *hlyA*, *espADB*, *tir*; C: *eaeA*, *hlyA*<sup>+</sup>, *espADB*, *tir*; D: *eaeA*, *hlyA*, *espADB*<sup>+</sup>, *tir*; E: *eaeA*, *hlyA*, *espADB*, *tir*<sup>+</sup>; F: *eaeA*, *hlyA*, *espADB*<sup>+</sup>, *tir*<sup>+</sup>; G: *eaeA*, *hlyA*, *espADB*<sup>+</sup>, *tir*<sup>+</sup>; H: *eaeA*<sup>+</sup>, *hlyA*<sup>+</sup>, *espADB*<sup>+</sup>, *tir*<sup>+</sup>.

### 3.4. Expression of *stx* genes in STEC O157 strains

To compare mRNA and protein expression of *stx1* and *stx2* genes, qPCR and rapid expression assay, RPLA were performed in the STEC O157 strains ( $n = 13$ ). Seven strains possessed *stx2* gene only and six strains carried both *stx1* and *stx2* genes. For each isolate, analysis of gene expression of *gapA*, *stx1*, and *stx2* was performed in the same PCR run. Application of qPCR assays indicated that the mRNA expressions of *stx2* gene were higher than those of *stx1* gene. Using dilution gradients of culture supernatant fluids by RPLA, expression titres of *stx* genes were given.

The expression titres of *Stx2* was also higher than those of *Stx1* (Table 5). However, in some strains, low expressions of *stx2* gene were found; for example, mRNA expression of Strain 2 and protein expressions of Strains 3 and 4.

## 4. Discussion

The present study employed a range of several *E. coli* organisms in the aetiology of diarrhoeal patients in Korea providing to the genetic characterization with regard to their harbouring of potential virulence genes.

To obtain the genetic and serological diversity of pathogenic *E. coli*, the association between the virulence factors and serotypes of isolates found in human was examined in this study. We showed that the genotypes of O157 serotype and non-O157 serotypes in STEC strains were different. The results indicated that all O157 serotypes of the STEC strains carried all the tested virulence genes, whereas these genes were detected in a lesser percent of the non-O157 STEC strains. Moreover, the *stx2* gene was present in the great majority of the O157. These crude data suggest an association of *stx2* with isolates of serotypes found in humans with severity of disease. O'Brien et al.<sup>19</sup> reported that 67 EHEC O157 strains tested possessed the EHEC *hlyA* gene. Boerlin et al.<sup>20</sup> reported that *eaeA* and *stx2* were significantly more frequent in isolates from serotypes found in humans with severe disease. It has been shown that the chromosomal virulence genes of EHEC and EPEC are organized as a cluster referred to as a pathogenicity island.<sup>5</sup> Our data showed that the genotype in STEC and EPEC was similar (Table 2). Genes for both classes are found predominantly on plasmids, and some ST-encoding genes have been found on transposon.<sup>2</sup> STa has about 50% identity to *east1* of EAEC. It has recently been reported that some strains of ETEC may also express *east1* in addition to STa.<sup>2,12</sup> STb

**Table 5** Expression of *stx* genes in Shiga toxin-producing *Escherichia coli* O157 strains

Strains	Genotype (PCR)		real-time RT PCR		RPLA titre	
	<i>stx1</i>	<i>stx2</i>	<i>stx1</i>	<i>stx2</i>	Stx1	Stx2
1	Neg.	Pos.	0	4738	0	256
2	Neg.	Pos.	0	5	0	0
3	Neg.	Pos.	0	3459	0	4
4	Neg.	Pos.	0	1499	0	4
5	Neg.	Pos.	0	3346	0	256
6	Neg.	Pos.	0	11831	0	256
7	Neg.	Pos.	0	2005	0	256
8	Pos.	Pos.	2740	5293	32	256
9	Pos.	Pos.	1098	4673	128	256
10	Pos.	Pos.	2320	5711	64	256
11	Pos.	Pos.	2135	6081	64	256
12	Pos.	Pos.	1323	2164	64	256
13	Pos.	Pos.	1370	2273	64	256

PCR = polymerase chain reaction; RT-PCR = reverse-transcriptase polymerase chain reaction; RPLA = reversed-passive latex agglutination; Neg. = negative; Pos. = positive; *stx* = Shiga toxin gene; Stx = Shiga toxin.

has been found only in ETEC.<sup>2</sup> The main conclusion of these previous investigations is that no single factor is responsible for the virulence of *E coli* strains.

The second part of our study showed differences of *stx* genes in O157 strains that are known as a pathogen in association with severe disease. Previous studies have shown that the virulence of STEC for humans may be related to the type of *stx*, which is produced by the bacteria and serotype.<sup>21</sup> In a study concerning Stx production as a single microbial factor, the most pathogenic strains for humans have been found to produce Stx2 only.<sup>2</sup> The Stx2 toxin has been described as being 1,000 times more cytotoxic than Stx1 towards human renal microvascular endothelial cells.<sup>2,8</sup> In other studies, Stx2 was found to be related with high virulence and was significantly associated with STEC strains from haemolytic-uremic syndrome patients.<sup>8</sup> Our data indicated that the expression of *stx* genes was different. Although low expressions of *stx2* gene were found in some strains (Table 5), we suggest that most O157 strains show more expression of *stx2* mRNA and protein than *stx1*. The low expression of *stx2* gene implies that the *stx* expression may be influenced by environmental conditions of each strain.

In conclusion, the present study demonstrates the diversity of virulence genes and serotypes in pathogenic *E coli* isolated from diarrhoea patients and the importance of *stx2* gene in the infection of STEC O157. Thus, it can provide the epidemiological information regarding the trend of STEC and non-STEC infections in the general population and show the fundamental data in association of serotypes with virulence genes in diarrhoeagenic *E coli* strains from Korea.

## Acknowledgements

This study was supported by a grant from Korea National Institute of Health, Seoul, Korea.

## References

- Clarke SC. Diarrhoeagenic *Escherichia coli*: an emerging problem? *Diagn Microbiol Infect Dis* 2001;**41**:93–9.
- Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998;**11**:142–201.
- Cho SH, Kim JH, Kim JC, et al. Surveillance of bacterial pathogens associated with acute diarrheal disease in the Republic of Korea during one year, 2003. *J Microbiol* 2006;**44**:327–35.
- Clarke SC, Haigh RD, Freestone PPE, Williams PH. Virulence of enteropathogenic *Escherichia coli*, a global pathogen. *Clin Microbiol Rev* 2003;**16**:365–78.
- Crepin VF, Junkal G, Frankel G. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. *Infect Immun* 2005;**73**:2573–85.
- Osek J. Development of a multiplex PCR approach for the identification of shiga toxin-producing *Escherichia coli* strains and their major virulence factor genes. *J Appl Microbiol* 2003;**95**:1217–25.
- Sjöling Å, Wiklund G, Savarino SJ, et al. Comparative analyses of phenotypic and genotypic methods for detection of enterotoxigenic *Escherichia coli* toxins and colonization factors. *J Clin Microbiol* 2007;**45**:3295–301.
- Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005;**365**:1073–86.
- Schmidt H, Zhang WL, Kohler B, et al. Genetic diversity of intimin genes of attaching and effacing *Escherichia coli* strains. *J Clin Microbiol* 2002;**40**:4486–92.
- Makino SI, Tobe T, Asakura H, et al. Distribution of the secondary type III secretion system locus found in enterohemorrhagic *Escherichia coli* O157:H7 isolates among shiga toxin-producing *E coli* strains. *J Clin Microbiol* 2003;**41**:2341–7.
- Taylor KA, O'Connell CB, Luther PW, Donnenberg MS. The EspB protein of enteropathogenic *Escherichia coli* is targeted to the cytoplasm of infected HeLa cells. *Infect Immun* 1998;**66**:5501–7.
- Dubreuil JD, Menard LP, Lussier JG, et al. Expression, purification, and biochemical characterization of enteroaggregative *Escherichia coli* heat-stable enterotoxin 1. *Prot Exp Puri* 2004;**33**:223–31.
- Nashikawa YZ, Hase A, Ogasawara J. Diarrheagenic *Escherichia coli* isolated from stools of sporadic cases of diarrheal illness in Osaka city, Japan between 1997 and 2000: prevalence of enterotoxigenic *E coli* heat-stable enterotoxin 1 gene-possessing *E. coli*. *Jpn J Infect Dis* 2002;**55**:183–90.
- Griffiths MW, Anand SK. Quorum sensing and expression of virulence in *Escherichia coli* O157:H7. *Int J Food Microbiol* 2003;**85**:1–9.
- Sperandio V, Torres AG, Giron JA, Kaper JB. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* 2001;**183**:5187–97.
- Walters M, Sperandio V. Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. *Infect Immun* 2006;**74**:5445–55.
- Kim JC, Yoon JW, Kim JB, et al. Omics-based analysis of the *luxS* mutation in a clinical isolate of *Escherichia coli* O157:H7 in Korea. *J Microbiol Biotechnol* 2010;**20**:415–9.
- Guinée PAM, Agterberg CM, Jansen WH. *Escherichia coli* O antigen typing by means of a mechanized microtechnique. *Appl Microbiol* 1972;**24**:127–31.
- O'Brien AD, Scott ME, Melton-Celsa AR. Mutations in *hns* reduce the adherence of Shiga toxin-producing *E coli* O91:H21 strain B2F1 to human colonic epithelial cells and increase the production of hemolysin. *Microb Pathog* 2003;**34**:155–9.
- Boerlin P, Schmid H, Burnens AP, et al. Verocytotoxin-producing *Escherichia coli* in patients with diarrhoea in Switzerland. *Eur J Clin Microbiol Infect Dis* 2002;**21**:810–3.
- Jenkins C, Chart H. Serodiagnosis of infection with verocytotoxin-producing *Escherichia coli*. *J Appl Microbiol* 1999;**86**:569–75.